0022-3565/09/3282-448–457$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 328:448–457, 2009

Vol. 328, No. 2
146464/3427164
Printed in U.S.A.

Novel, Potent, and Selective $GABA_C$ Antagonists Inhibit Myopia Development and Facilitate Learning and Memory

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Received September 22, 2008; accepted November 3, 2008

ABSTRACT

This study reports pharmacological and physiological effects of *cis*- and *trans*-(3-aminocyclopentanyl)butylphosphinic acid (*cis*- and *trans*-3-ACPBPA). These compounds are conformationally restricted analogs of the orally active $GABA_{B/C}$ receptor antagonist (3-aminopropyl)-*n*-butylphosphinic acid (CGP36742 or SGS742). *cis*- [$IC_{50}(\rho1) = 5.06\ \mu M$ and $IC_{50}(\rho2) = 11.08\ \mu M$; $n = 4$] and *trans*-3-ACPMPA [$IC_{50}(\rho1) = 72.58\ \mu M$ and $IC_{50}(\rho2) = 189.7\ \mu M$; $n = 4$] seem competitive at $GABA_C$ receptors expressed in *Xenopus laevis* oocytes, having no effect as agonists (1 mM) but exerting weak antagonist (1 mM) effects on human $GABA_A$ and $GABA_B$ receptors. *cis*-3-ACPBPA was more potent and selective than the *trans*-compound, being more than 100 times more potent at $GABA_C$ than $GABA_A$ or $GABA_B$ receptors. *cis*-3-ACPBPA was further evaluated on dissociated rat retinal bipolar cells and dose-dependently inhibited the native $GABA_C$ receptor ($IC_{50} = 47 \pm 4.5\ \mu M$; $n = 6$). When applied to the eye as intravitreal injections, *cis*- and *trans*-3-ACPBPA prevented experimental myopia development and inhibited the associated vitreous chamber elongation, in a dose-dependent manner in the chick model. Doses only 10 times greater than required to inhibit recombinant $GABA_C$ receptors caused the antimyopia effects. Using intraperitoneal administration, *cis*- (30 mg/kg) and *trans*-3-ACPBPA (100 mg/kg) enhanced learning and memory in male Wistar rats; compared with vehicle there was a significant reduction in time for rats to find the platform in the Morris water maze task ($p < 0.05$; $n = 10$). As the physiological effects of *cis*- and *trans*-3-ACPBPA are similar to those reported for CGP36742, the memory and refractive effects of CGP36742 may be due in part to its $GABA_C$ activity.

GABA is an abundant neurotransmitter that mediates inhibition throughout the retina and central nervous system. Three main classes of GABA receptors exist and are termed $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Bormann, 2000; Chebib and Johnston, 2000). The $GABA_A$ and $GABA_C$ are ionotropic receptors, belonging to the Cys-loop family of ligand-gated ion channels, which includes nicotinic acetylcholine, strychnine-sensitive glycine, serotonin type 3, and some invertebrate anionic glutamate receptors (Bormann, 2000; Chebib and Johnston, 2000). Both $GABA_A$ and $GABA_C$ receptors are chloride channels that mediate fast synaptic inhibition when activated by GABA. In contrast, $GABA_B$ receptors are members of the metabotropic receptor family; these receptors couple via G proteins ($G_{i/o}$) to interact with neuronal inwardly rectifying potassium and voltage-gated calcium channels, mediating slow synaptic inhibition by increasing

This work was supported in part by the National Institutes of Health [Grant EY12028], Circadian Technologies Pty. Ltd., and the Institute of Health and Biomedical Innovation.

This article contains data presented in part in Schmid KL, Brinkworth D, Wallace KM, Payer R, Fritsch C, and Lambrou GN (2004) The effects of GABA-B and GABA-C antagonists on myopia development in chick, in *Proceedings of the 10th International Conference on Myopia*; 2004 July 19–22; Cambridge, UK; and Schmid KL, Brinkworth DR, Chebib M, Hanrahan JR, and Johnston GAR (2005) A theoretical model for GABAc antagonists inhibiting myopia via the ON/OFF retinal pathway, in *Proceedings of the Joint Meeting of Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and Australasian Pharmaceutical Science Association*; 2005 December; Melbourne, Australia.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.108.146464.

ABBREVIATIONS: TPMPA, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid; 3-APMPA, (3-aminopropyl)-methylphosphinic acid; *cis*- and *trans*-3-ACPMPA, *cis*- and *trans*-(3-aminocyclopentanyl)methylphosphinic acid; *cis*- and *trans*-3-ACPBPA, *cis*- and *trans*-(3-aminocyclopentanyl)butylphosphinic acid; CGP36742 or SGS742, (3-aminopropyl)-*n*-butylphosphinic acid; GIRK, G protein-coupled inwardly rectifying potassium channel; D, diopter.

potassium and decreasing calcium conductances (Marshall et al., 1999).

The $GABA_A$ receptor consists of a heteropentomeric composition of the following subunits: α_{1-6}, β_{1-4}, γ_{1-3}, δ, ε, π, and θ. A functional receptor usually requires two α-, two β-, and one other type of subunit (e.g., $\alpha_1\beta_2\gamma_{2L}$; Chebib and Johnston, 2000). The $GABA_B$ receptor represents a novel type of G protein-coupled receptor in that they are heterodimers (Marshall et al., 1999). This receptor is believed to be composed of two subunits, a ligand-binding subunit ($GABA_B1$) and a signal transduction subunit ($GABA_B2$) (Marshall et al., 1999).

The $GABA_C$ receptor consists of homo-oligomeric or pseudohomo-oligomeric composition of five subunits from the rho (ρ) subunit family and cloned from human, rat, perch, and chick (Bormann, 2000; Chebib and Johnston, 2000). The $GABA_C$ receptor has a distinct pharmacology to that of $GABA_A$ and $GABA_B$ receptors: they are not inhibited by the alkaloid bicuculline or affected by barbiturates and benzodiazepines, which characteristically affect $GABA_A$ receptors. Furthermore, $GABA_C$ receptors are not activated by (*R*)-(−)-baclofen or inhibited by (*R*)-(−)-phaclofen, which typically act at $GABA_B$ receptors (Chebib and Johnston, 2000). The $GABA_C$ receptors are selectively activated by (+)-*cis*-2-aminomethylcyclopropanecarboxylic acid (Duke et al., 2000) and blocked by (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) (Ragozzino et al., 1996).

$GABA_C$ receptors have been implicated in ammonia-induced apoptosis in hippocampal neurons (Yang et al., 2003) and in regulating hormone release in the pituitary gland (Boue-Grabot et al., 2000). Furthermore, TPMPA has been shown to 1) enhance memory in chicks (Gibbs and Johnston, 2005); 2) modulate the sleep-waking behavior in rats (Arnaud et al., 2001); 3) inhibit synaptic transmission in the neonatal rat spinal cord in vitro (Rozzo et al., 1999); and 4) inhibit myopia development in chicks (Stone et al., 2003), indicating possible role for $GABA_C$ receptors in memory, circadian rhythms, and myopia. However, TPMPA does not cross the blood-brain barrier because no central effects upon systemic administration have been reported to date. To understand the role of $GABA_C$ receptors in the brain, a potent, selective ligand that can cross the blood-brain barrier is required.

Recently, we developed *cis*- and *trans*-(3-aminocyclopentanyl)methylphosphinic acid (*cis*- and *trans*-3-ACPMPA) from a structure-function study of various cyclopentane analogs of GABA (Chebib et al., 2001, 2007; Hanrahan et al., 2006). These conformationally restricted methyl phosphinic acids are highly potent and selective for ρ1 $GABA_C$ receptors, being greater than 100 times more potent at $GABA_C$ than $GABA_A$ receptors and 50 times more potent at $GABA_C$ than $GABA_B$ receptors. Although, *cis*- and *trans*-3-ACPMPA seem to be less selective for $GABA_C$ receptors than TPMPA (Ragozzino et al., 1996), they remain important pharmacological leads for the design and development of selective $GABA_C$ receptor ligands, with potential physiological activity. Because *cis*- and *trans*-3-ACPMPA are restricted analogs of the straight-chain $GABA_B$ agonist and $GABA_C$ antagonist (3-aminopropyl)-methylphosphinic acid (Froestl et al., 1995), the numbers of conformations that bind to the $GABA_B$ compared with the $GABA_C$ receptors are reduced.

The aim of this study was to evaluate the pharmacological and physiological effects of the *n*-butyl phosphinic acid analogs of *cis*- and *trans*-3-ACPMPA, *cis*- and *trans*-(3-aminocyclopentanyl)-*n*-butylphosphinic acid (*cis*- and *trans*-3-ACPBPA; Fig. 1). These compounds are conformationally restricted analogs of the orally active $GABA_{B/C}$ receptor antagonist (3-aminopropyl)-*n*-butylphosphinic acid (CGP36742 or SGS742) (Froestl et al., 1995). CGP36742 increases spatial memory in rodents (Carletti et al., 1993; Helm et al., 2005) and inhibits myopia progression in chicks (Schmid et al., 2004). However, it is not clear whether the physiological effects of CGP36742 are due to its $GABA_B$ or $GABA_C$ activity. This study shows that *cis*- and *trans*-3-ACPBPA are selective $GABA_C$ receptor antagonists with effects on the development of myopia after intravitreal administration and on learning and memory after systemic administration.

Materials and Methods

This research was aimed at 1) evaluating the effects of *cis*- and *trans*-3-ACPBPA in vitro using recombinant human GABA receptors expressed in *Xenopus laevis* oocytes and on native $GABA_C$ receptors expressed on rat retinal bipolar cells; and 2) evaluating the in vivo effects on myopia in chick and on learning and memory in rat. All experiments were conducted with ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, published by the National Health and Medical Research Council of Australia.

Drugs and DNA Sources. *cis*- and *trans*-3-ACPBPA were synthesized according to our previously published methods (Hanrahan et al., 2006). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise stated. Human ρ1 cDNA encapsulated in the pcDNA1.1 vector (Invitrogen, Carlsbad, CA) was donated by Dr. George Uhl (National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD). Human ρ2 cDNA encapsulated in the pKS vector was kindly donated by Dr. Garry Cutting (Center for Medical Genetics, John Hopkins University, School of Medicine, Baltimore, MD). Human α_1, β_2, and γ_{2L} $GABA_A$ cDNAs encapsulated in pcDM8 were gifts from Dr. Paul Whiting (Merck Sharpe and Dohme, Hoddesdon, UK). Human $GABA_{B(1b)}$, $GABA_{B2}$ cDNA and rat G protein-coupled inwardly rectifying potassium channels (GIRK) 1 and 4 were provided by Dr. Andrew Green (GlaxoSmithKline, Uxbridge, Middlesex, UK). Human $GABA_{B(1b)}$ was encapsulated in the pcDNA3.1(−) (Invitrogen), $GABA_{B2}$ and rat GIRK1 were encapsulated in the pcDNA3 (Invitrogen), whereas the rat GIRK4 was encapsulated in pBluescript KS(−) (Stratagene, La Jolla, CA).

Isolation and Purification of cDNA and mRNA. ρ1, ρ2, α_1,β_2,γ_{2L}, $GABA_{B(1b)}$, $GABA_{B2}$, GIRK1, and GIRK4 cDNAs encapsu-

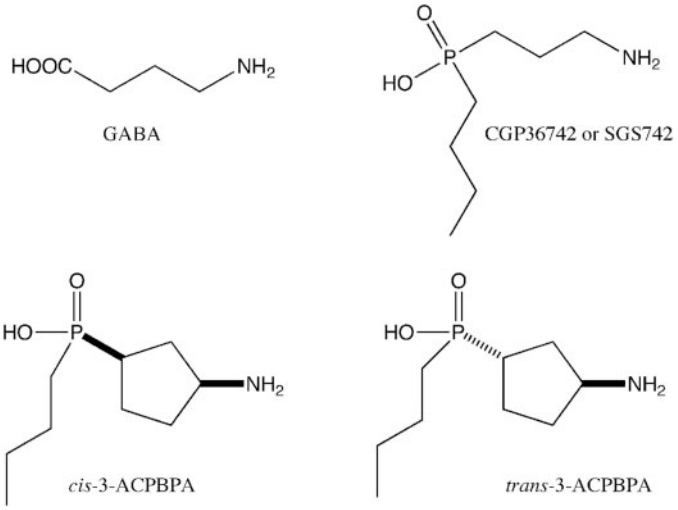

Fig. 1. Structure of $GABA_C$ ligands.

lated in the relevant plasmid vector were linearized with the restriction endonuclease as reported previously (see Table 1 for the list of cDNAs and corresponding endonucleases). Linearized cDNA were transcribed to mRNA using the T7 mMESSAGE mMACHINE kit (Ambion, Austin, TX).

Expression of GABA Receptors in *X. laevis* Oocytes. Electrophysiological methods were performed as described previously (Chebib et al., 1997). In brief, oocytes were harvested from *X. laevis* (housed in the Department of Veterinary Science at the University of Sydney, Sydney, NSW, Australia) and defolliculated. The oocytes were then stored in ND96 solution: 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES (hemi-sodium salt) supplemented with 2.5 mM sodium pyruvate, 0.5 mM theophylline, and 50 μg/ml gentamicin.

Stage V to VI oocytes were injected with 50 nl containing 10 ng of mRNA and then stored at 16°C. Recordings of receptor activity were obtained after 2 to 8 days by a two-electrode voltage clamp by means of a GeneClamp 500 amplifier (Axon Instruments, Foster City, CA), a MacLab 2e recorder (ADInstruments Pty Ltd., Sydney, NSW, Australia), and Chart version 3.6.3 program (ADInstruments). Oocytes were voltage-clamped at −60 mV using glass electrodes filled with 3 mM KCl (0.5–1.5 MΩ). The preparation was continually perfused with ND96 solution at room temperature. Known concentrations of ligands dissolved in either ND96 or 45 mM K^+ buffer [45 mM NaCl, 45 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES (hemi-sodium salt)] were applied in the absence and presence of GABA, respectively, until maximal current was reached, at which time the oocyte was washed for 5 to 10 min to allow complete recovery of response to a known maximal dose of GABA. In the case of antagonists, two dose-response curves were conducted on the same cell: a GABA dose-response curve and GABA dose-response curve in the presence of a known concentration of antagonist. All compounds were tested on oocytes from at least three harvests.

The current elicited by each drug on expressed receptors was measured and standardized to the maximum GABA activated current on the cell. For determining the IC_{50} values of *cis*-3- and *trans*-3-ACPBPA on ρ1 and ρ2, the concentration of GABA used was 1 μM. The current evoked by each compound was standardized to current evoked by GABA in the following ratio (I/I_M) unless otherwise stated. The dose-response curves were plotted using the current ratios versus concentration. Dose-response curves [sigmoidal dose-response (variable slope)] were obtained using the least-squares regression equation $I/I_M = \text{bottom} + (\text{top} - \text{bottom})/[1 + 10^{((\log EC_{50} \text{ (or } \log IC_{50}) - \log[A]) \times n_H)}]$, where I is the current at a known concentration of agonist; I_M is the maximal current; [A] is the agonist (GABA) concentration; EC_{50} is concentration of GABA that activates 50% of receptors and IC_{50} is the concentration of antagonist that inhibits 50% of receptors at a given agonist concentration, and n_H is the Hill coefficient. This equation is identical to the four-parameter logistic equation, where bottom refers to the estimated response at zero concentration and top refers to estimated response at infinite concentration (Prism 4; GraphPad Software Inc., San Diego, CA).

Cell Isolation and Patch-Clamp Recording. Solitary bipolar cells were isolated from the rat retina according to published protocols (Ramsey et al., 2006). In brief, rats were euthanized in a CO_2 chamber, eyes were enucleated and hemisected, and the retinas gently removed from the posterior eyecup and immersed for 40 min in a modified Ame's media (supplemented with 0.88 g/l NaCl, 2.36 g/l HEPES, and 10,000 units/l penicillin/streptomycin, pH 7.4) containing 2 mg/ml papain (EMD Biosciences, San Diego, CA) and 1 mg/ml *l*-cysteine (Sigma-Aldrich). After several brief washes, the tissue was triturated through a sterile pipette, and aliquots of the supernatant containing dissociated cells were placed in culture dishes with modified Ame's medium. The cells were maintained at room temperature for up to 8 h. Before recording, the culture medium was replaced with an extracellular solution consisting of 120 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM dextrose, pH 7.4. Whole-cell membrane currents were recorded with a patch pipette filled with an intracellular solution containing 130 mM CsCl, 4 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 11 mM EGTA, 10 mM HEPES, 1 mM MgATP, and 0.2 mM NaGTP, pH 7.4. Cells were held at −60 mV, and the GABA-elicited responses were determined on an Axopatch 200B amplifier controlled by pCLAMP software (Axon instruments). The concentration of GABA used to determine the IC_{50} value of *cis*-3-ACPBPA was 10 μM. This concentration gives a submaximal response for $GABA_C$ receptors and provides an optimal signal for the experiment (Ramsey et al., 2006). Data were plotted using Origin Software (OriginLab Corp., Northampton, MA).

Evaluating the Effects of *cis*- and *trans*-3-ACPBPA on Myopia in Chick. Rhode Island Red-Rhode Island White cross cockerels ($n = 74$) were obtained on the day of hatching from a local supplier (Bond Nelbex, Brisbane, QLD, Australia). On day 8 after hatch chicks were fitted monocularly with a −15 D spectacle lens (Schmid and Wildsoet, 1996). Chicks ($n = 7$–10/treatment group) were assigned to one of three drug treatments: 1) water control (sterile injectable water), 2) *cis*-3-ACPBPA, or 3) *trans*-3-ACPBPA. Four doses of *cis*- and *trans*-3-ACPBPA [0.35 (78 ng) to 350 nmol (78 μg) in $\log_{10}$ steps] were tested. All drugs were delivered daily as 10-μl intravitreal injections, as described previously (Schmid and Wildsoet, 2004). Doses were calculated to be within the physiologically active range using known receptor affinities (determined under Cell Isolation and Patch-Clamp Recording and Analysis of Electrophysiological Data) and assuming an average vitreous chamber volume of 150 μl (for details, see Schmid and Wildsoet, 2004). In all treatment groups, fellow eyes were left untreated (no visual manipulation and no drug treatment) for comparative purposes.

After 4.5 days of lens wear and four drug applications, measurements of refractive error and axial ocular dimensions were made by streak retinoscopy and A-scan ultrasonography, respectively, under 2% isoflurane anesthesia (for details, see Schmid et al., 2006). Data presented are mean ± S.D. interocular differences (treated minus untreated eye data), unless otherwise specified. Statistical analysis comprised factorial analyses of variance and Tukey's post hoc tests, which were carried out using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL).

General Behavioral and Acute Toxicity Tests in Mice. The overt behavioral effects of *cis*- and *trans*-3-ACPBPA on mice after intraperitoneal injection were assessed using the protocol described by Irwin (1968). Tests were performed on male QS mice aged 10 weeks, weighing 35.5 g on average. Animals were handled on five occasions before experimentation to habituate them to handling. Animal's tails were marked with waterproof marker to assign them to one of four groups, each consisting of four animals for random, blind allocation to one of four experimental conditions: vehicle, test dose 1, test dose 2, and test dose 3, which were 1, 10, and 100 mg/kg. Drugs were administered intraperitoneally using a vehicle of 10% propylene glycol in 0.9% saline.

Observations were recorded pertaining to the following 34 parameters: spontaneous activity, exophthalmics, piloerection, aggressiveness, writhing, tremors, clonic convulsions, tonic convulsions, gasping, hypersensitivity, docility, position struggle, salivation, cyanosis, vasoconstriction, vasodilation, finger approach, finger escape, hind limb placing, ataxia, hyperesthesia, Straub tail, visual placing, positional, tail pinch, righting reflex, traction, ptosis, corneal reflex, pinna reflex, catalepsy, bizarre behavior, prehensile strength, and pupil size according to the procedures described by Irwin (1968).

Evaluating the Effects of *cis*- and *trans*-3-ACPBPA on Rat Learning and Memory in the Morris Water Maze. The Morris

TABLE 1
List of cDNAs with corresponding restriction endonuclease used

cDNA	Enzyme	cDNA	Enzyme	cDNA	Enzyme
ρ1	Xba1	GIRK4	Xba1	GIRK1	NOT1
β2	NOT1	γ2L	NOT1	$GABA_{B(1b)}$	BAMH1
$GABA_{B2}$	Xba1	α1	NOT1	ρ2	ECO RV

water maze experiments were performed by the Centre de Recherches Biologiques (Baugy, France) under contract. In brief, groups of 10 male adult Wistar rats, weighing between 195 and 281 g on the day of administration [i.e., day (D) 1], were obtained from the Dépré Breeding Center (Saint Doulchard, France). Testing of spatial memory involved three successive steps: training in the Morris water maze on D0 followed by 2 days of testing (D1 and D2). On D1, drug(s) or vehicle control was given 30 min before testing. No treatment was given on D2. Animals were not fasted before performing the task.

For training (D0), the animals were placed in the water pool with the platform in the middle of the pool. The rat was allowed to explore the pool for 20 s, and if it did not locate the platform, it was helped to reach it and to climb onto it. The animal was left for 30 s on the platform, and it was then returned to its housing. The goal of the training period on D0 is to show to the animal that there is an escape possibility, which is the platform. This training is required to decrease the heterogeneity on the first day of the test, D1.

On D1, animals were randomly given the vehicle (saline) ($n = 10$), scopolamine (1 mg/kg; $n = 10$), *cis*- (10, 30, and 100 mg/kg; $n = 10$ for each dose), or *trans*-3-ACPBPA (10, 30, and 100 mg/kg; $n = 10$ for each dose) by intraperitoneal injection in a volume of 1 ml/kg. On this day, the learning and not memory capacity of the animals is evaluated. The platform was placed in the northeastern quarter of the pool (to avoid any memory phenomenon from the previous day), and 30 min after dosing (to allow time for the drug to act), the rat was placed in the most southern point of the pool. A videocamera was used to measure the time it took the rat to locate the platform and to also score the behavior of the rat within the pool. If the animal did not locate the platform within 90 s, it was assisted to find and climb the platform. Three consecutive trials were performed for each animal. In these trials, animals were placed directly in the pool at the south point and observed for 90 s, and each sequence for one animal was performed at 15- to 20-min intervals.

On D2, no treatment was performed, and effects on memory were measured. Four trials were performed according to the same procedure as described on D1 but with different starting points [trial 1, south (as in D1); trial 2, southwest; trial 3, west; and trial 4, southeast]. The number of animals to reach the platform unaided was recorded together with the time in seconds it took for the animal to locate the platform. Therefore, the more sensitive trials to detect any effects of the drug are trials 1 and 2 on D1 and D2. The behavior of each animal in the pool was graded according to the scale described in Table 2. A high number indicates good performance and a low number poor performance. Statistical analysis was performed using the nonparametric Mann-Whitney U test with unilateral comparison.

TABLE 2
Behavior in the pool

Grade	Observation
0	The animal stays put and tries to climb on the side of the pool.
1	The animal swims around the pool and tries frequently to climb on the side of the pool.
2	The animal swims around the pool and tries to climb on the side of the pool but sometimes swims through the pool.
3	The animal swims around the pool less frequently; it swims through the pool for most of the time but does not find the platform.
4	The animal swims around the pool less frequently; it swims through the pool for most of the time and finds the platform.
5	The animal swims in the quarter where the platform is but does not find it.
6	The animal swims in the quarter where the platform is and finds it
7	At the beginning, the animal stays put and floats. Subsequently, it swims directly to the platform.
8	The animal swims directly to the platform.

Results

We found that *cis*- and *trans*-3-ACPMPA were potent and selective antagonists at the ρ1 and ρ2 $GABA_C$ receptor. Both compounds prevented experimental myopia development and both enhanced learning and memory in the Morris water maze.

Effects of *cis*- and *trans*-3-ACPBPA on Recombinant $GABA_A$ and $GABA_B$ Receptors. Figure 2A shows an example of a cell injected with $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$) mRNA, illustrating a response to a maximal dose of GABA (300 μM). Both *cis*- (1 mM) and *trans*-3-ACPBPA (1 mM) had no response alone. However, when *cis*-3-ACPBPA (1 mM) was applied in the presence of an approximate EC_{50} concentration of GABA (60 μM), the response of GABA (60 μM) was reduced by 33 ± 3% ($n = 3$), indicating weak antagonist effects. Likewise, *trans*-3-ACPBPA (1 mM) was then administered in the presence of an EC_{50} concentration of GABA (60 μM), and it reduced the response by 28 ± 5% ($n = 3$), indicating weak antagonist effects.

Figure 2B shows an example of a cell injected with $GABA_{B(1b)}$, $GABA_{B2}$, GIRK1, and GIRK4 mRNAs. Upon exposure to 45 mM K^+ solution, the GIRK1/4 channels are stimulated. There was a further response to an application of a maximal (100 μM) and an EC_{50} (3 μM) concentration of GABA. No response was observed when *cis*- (1 mM) or *trans*-3-ACPBPA (1 mM) was applied alone. However, in the presence of GABA (3 μM), *cis*- (1 mM) and *trans*-3-ACPBPA (1 mM) inhibited the response of GABA by 27 ± 3 and 35 ± 5%, respectively ($n = 3$).

Figure 2C shows the activity of a maximal and submaximal dose of GABA (100 and 1 μM, respectively), TPMPA (30 μM), *cis*-3-ACPBPA (40 μM), and *trans*-3-ACPBPA (30 μM) at ρ1 $GABA_C$ receptors. *cis*- and *trans*-3-ACPBPA had no effect alone. In the presence of GABA (1 μM), TPMPA and *cis*-3-ACPBPA completely inhibited the response, whereas *trans*-3-ACPBPA inhibited the response by 70% (Fig. 2C).

Effects of *cis*- and *trans*-3-ACPBPA on Recombinant ρ1 and ρ2 $GABA_C$ Receptors. The inhibitory effects of *cis*- and *trans*-3-ACPBPA were further evaluated against GABA (1 μM) at ρ1 and ρ2 $GABA_C$ receptors, and these results are summarized in Table 3 and shown in Fig. 3. The IC_{50} values for *cis*-3-ACPBPA at ρ1 and ρ2 $GABA_C$ receptors were 5.06 μM (log $IC_{50} = 0.704 \pm 0.025$) and 11.08 μM (log $IC_{50} = 1.044 \pm 0.086$), respectively. The IC_{50} values for *trans*-3-ACPBPA at ρ1 and ρ2 $GABA_C$ receptors were 72.58 μM (log $IC_{50} = 1.861 \pm 0.043$) and 189.7 μM (log $IC_{50} = 2.278 \pm 0.034$), respectively. Both *cis*- and *trans*-3-ACPBPA seemed to be competitive antagonists at ρ1 $GABA_C$ receptors over the concentration tested (Fig. 4). It is worthy to note that the methyl analogs *cis*- and *trans*-3-ACPMPA were found to be competitive antagonists at these receptors (Chebib et al., 2007).

Effects of *cis*-3-ACPBPA on Dissociated Rat Retinal Bipolar Cells. Isolated retinal bipolar cells were selected by their typical morphology (Qian and Dowling, 1995). Both $GABA_A$ and $GABA_C$ receptors are present on rat retinal bipolar cells (Euler and Wässle, 1998; Ramsey et al., 2006). Bicuculline (100 μM) was used to suppress the $GABA_A$ receptor activity on these cells. As an example shown in Fig. 5A, in the presence of bicuculline, 10 μM GABA elicited sustained responses on rat bipolar cells, reflecting the $GABA_C$ receptor activity on these cells. The $GABA_C$ receptor-mediated response could be inhibited by *cis*-3-ACPBPA in a concentration-dependent manner.

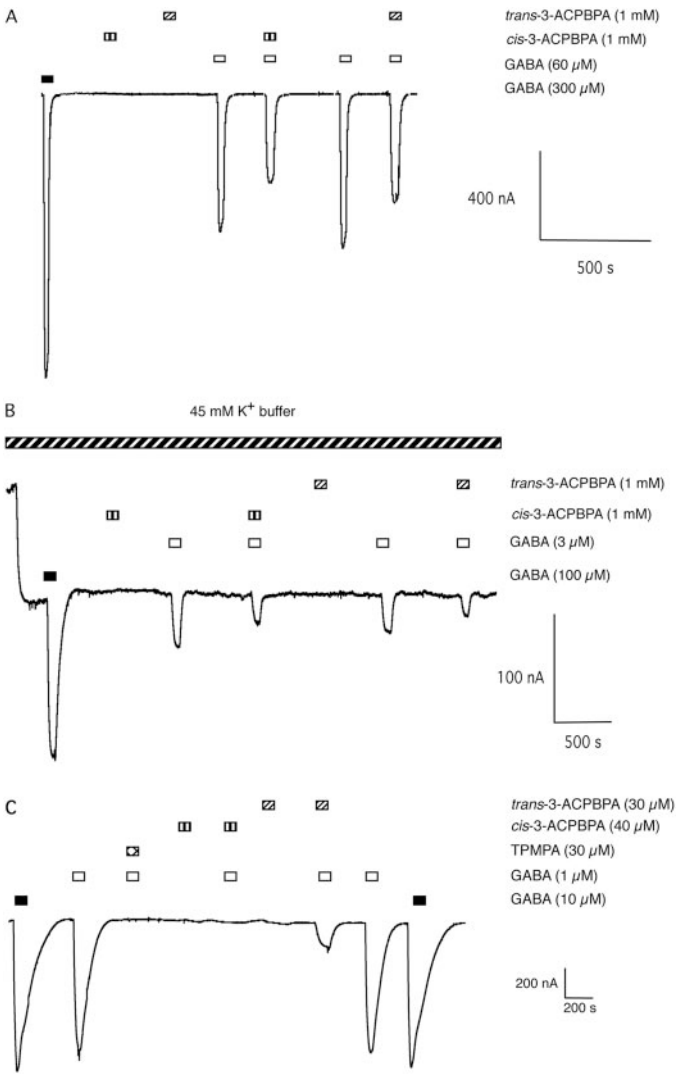

Fig. 2. A, GABA (300 μM) (duration indicated by black bar) activated a maximum current in oocytes expressing human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors clamped at −60 mV. *cis-* (1 mM; duration indicated by the vertically hatched bar) and *trans*-3-ACPBPA (1 mM; duration indicated by forward hatched bar) did not activate the receptor when applied alone. GABA (60 μM) (duration indicated by open bar) activated an inward current approximately 50% of the maximal current produced by GABA (300 μM). When coapplied with GABA (60 μM), *cis*-3-ACPBPA (1 mM, duration indicated by the vertically hatched bar) reduced the current by 33 ± 3% (n = 3). When coapplied with GABA (60 μM) *trans*-3-ACPBPA (1 mM; duration indicated by forward hatched bar) reduced the current by 28 ± 5% (n = 3). B, GABA (100 μM) (duration indicated by black bar) activated a maximum current in oocytes expressing human $GABA_{B(1b,2)}$ receptors clamped at −60 mV. *cis-* (1 mM; duration indicated by the vertically hatched bar) and *trans*-3-ACPBPA (1 mM; duration indicated by forward hatched bar) did not activate the receptor when applied alone. GABA (3 μM) (duration indicated by open bar) activated approximately 50% of the maximal current of GABA (100 μM). When coapplied with GABA (3 μM), *cis*-3-ACPBPA (1 mM, duration indicated by the vertically hatched bar) reduced the current by 27 ± 3% (n = 3). When coapplied with GABA (3 μM) *trans*-3-ACPBPA (1 mM, duration indicated by forward hatched bar) reduced the current by 35 ± 5% (n = 3). C. GABA (10 μM) (duration indicated by black bar) activated a maximal inward current in oocytes expressing human ρ1 $GABA_C$ receptors when clamped at −60 mV. GABA (1 μM) (duration indicated by open bar) activated an inward current 80 to 90% of the maximal current produced by GABA (10 μM). When TPMPA (30 μM; duration indicated by checked bar) is coapplied with GABA (1 μM), there is complete inhibition of the GABA response. Likewise, *cis*-3-ACPBPA (40 μM; duration indicated by the striped bar) did not activate a current. When coapplied with GABA (1 μM), *cis*-3-ACPBPA (40 μM; duration indicated by striped bar) completely reduced the GABA response. *trans*-3-ACPBPA (30 μM; duration indicated by the forward hatched bar) did not activate a current. When coapplied with GABA (1 μM), *trans*-3-ACPBPA (30 μM; duration indicated by forward hatched bar) reduced the GABA response by 70%.

Examples of the current responses elicited from a rat bipolar cell by coapplication of GABA (10 μM) and various concentrations of *cis*-3-ACPBPA are illustrated in Fig. 5A. The averaged dose-inhibition relation is shown in Fig. 5B. The data could be fitted by a Hill equation, with an IC_{50} value of 47 ± 4.5 μM and Hill coefficient of 0.97.

Effects of *cis-* and *trans*-3-ACPBPA on Lens-Induced Myopia. Negative spectacle lenses induce hyperopic defocus and when applied to infant animal's eyes, the eye develops myopia to compensate for the induced refractive error (Schmid and Wildsoet, 1996). The −15 D lens induced an average −11.8 ± 3.9 D of myopia, 0.46 ± 0.10 mm of vitreous chamber expansion, and 0.57 ± 0.13 mm of axial ocular expansion in water-injected control chicks. The ocular effects of the −15 D lens were significantly inhibited by both the *cis*-3-ACPBPA (refractive error: $F_{4,40} = 11.814$, $p < 0.0005$; vitreous chamber: $F_{4,40} = 11.930$, $p < 0.0005$; axial length $F_{4,40} = 9.992$, $p < 0.0005$) and *trans*-3-ACPBPA (refractive error: $F_{4,42} = 5.439$, $p < 0.005$; vitreous chamber: $F_{4,42} = 13.805$, $p < 0.0005$; axial length $F_{4,42} = 8.549$, $p < 0.0005$) intravitreal drug treatments. The ocular effects of both ACPBPA compounds followed a typical dose response function, with greater myopia inhibition at higher compared with lower test doses (Fig. 6). For *cis*-3-ACPBPA the two highest doses tested (35 and 350 nmol) significantly inhibited the myopia, vitreous chamber expansion, and axial expansion compared with water-injected animals ($p < 0.005$ for all comparisons and both doses). For *trans*-3-ACPBPA, the effects of the −15 D lens were only significantly inhibited at the highest dose tested (350 nmol, $p < 0.005$ for all comparisons). For the 350-nmol dose, myopia was reduced to an average −2.3 ± 2.6 D (80% myopia reduction) and −3.1 ± 2.8 D (74% reduction) and axial expansion to 0.17 ± 0.17 mm (70% reduction) and 0.15 ± 0.15 mm (74% reduction) for *cis-* and *trans*-3-ACPBPA, respectively.

Effects on General Behavioral and Acute Toxicity in Mice. *cis-* and *trans*-3-ACPBPA were tested at doses of 1, 10, and 100 mg/kg. Overt behavioral effects were observed for each mouse at 0, 30, and 60 min, 24, 48, and 72 h and 1 week after injection. No overt acute toxicity was observed with either isomer at any time point as judged from the observed lack of convulsions, respiratory distress (cyanosis, gasping), writhing, changes to reflex activity or mortality. The *cis*-isomer produced no overt behavioral effects at the doses tested. The *trans*-isomer produced only mild behavioral effects shortly after injection and 30 min after injection of 10 and 100 mg/kg in two of the four animals injected at each dose, and persisting in one animal when tested 60 min after injection of 100 mg/kg. These effects were observed as increased hypersensitivity (measured as increased irritability, reactivity, and aggressiveness during handling and toward cage mates), increased escape behavior (measured by general observation and finger escape behavior), biting behavior and decreased tolerance to handling, tests of docility, and corneal and pinna reflexes. At 30 min after injection, 100 mg/kg also increased spontaneous activity in the same two of four animals. At 24 h to 1 week after injection all animals seemed

TABLE 3

Effect of *cis*- and *trans*-3-ACPBPA at GABA receptors expressed in *X. laevis* oocytes

The IC_{50} values of *cis*- and *trans*-3-ACPBPA on $GABA_C\rho_1$ and ρ_2 receptors expressed in *X. laevis* oocytes using the concentration of GABA as 1 μM are shown. The values in parentheses are log IC_{50} ± S.E.M.

Compound	$GABA_A(\alpha_1\beta_2\gamma_{2L})$[a]	$GABA_{B(1b,2)}$[b]	IC_{50}	
			$GABA_C\rho_1$[c]	$GABA_C\rho_2$[c]
	%		μM	
cis-3-ACPBPA	33 ± 3	27 ± 3	5.062 (0.7043 ± 0.025)	11.08 (1.044 ± 0.086)
trans-3-ACPBPA	28 ± 5	35 ± 5	72.58 (1.861 ± 0.043)	189.7 (2.278 ± 0.034)

[a] Percentage inhibition *cis*-3-ACPBPA (1 mM) and *trans*-3-ACPBPA (1 mM) against an EC_{50} concentration of GABA (60 μM) at $\alpha_1\beta_2\gamma_{2L}$ expressed in *X. laevis* oocytes.
[b] Percentage inhibition *cis*-3-ACPBPA (1 mM) and *trans*-3-ACPBPA (1 mM) against an EC_{50} concentration of GABA (3 μM) at $GABA_{B(1b,2)}$ coupled to GIRK1 and GIRK4 expressed in *X. laevis* oocytes.

well, with no observable changes in behavior, spontaneous activity, or appearance.

Effects of *cis*- and *trans*-3-ACPBPA on Learning and Memory in the Morris Water Maze. The results of the water maze tests are shown in Fig. 7, and a summary of results by *cis*- and *trans*-3-ACPBPA against vehicle from trial 1 on D1 and D2 are highlighted in Table 4. Under the experimental conditions adopted, an intermediate dose of 30 mg/kg *cis*-3-ACPBPA administered by the intraperitoneal route significantly decreased the time taken for the rat to locate the platform, increased the number of rats that found the platform and produced a higher behavior score in the pool on D1 of testing ($p < 0.01$ compared with vehicle; Table 4; Fig. 7). On D2, rats dosed on D1 with *cis*-3-ACPBPA (30 mg/kg) maintained the ability to reach the platform significantly more quickly than the control animals (Table 4; Fig. 7; $p < 0.05$), but the behavior score in the pool did not differ. Despite a lower median time to find the platform in rats treated with 10 mg/kg during trial 1 on D1, this was not significant because the score was not different compared with vehicle. Furthermore, no significant effects were observed on D2 at doses of 10 or 100 mg/kg ($p > 0.05$) of the *cis*-compound compared with vehicle (Fig. 7).

Reflecting the different potency, a higher dose of *trans*-3-ACPBPA, the highest dose of 100 mg/kg *trans*-3-ACPBPA administered by the intraperitoneal route, significantly decreased the time taken for the rats to locate the platform on D1 (Table 4; Fig. 7; $p < 0.05$). On D2, a statistically signifi-

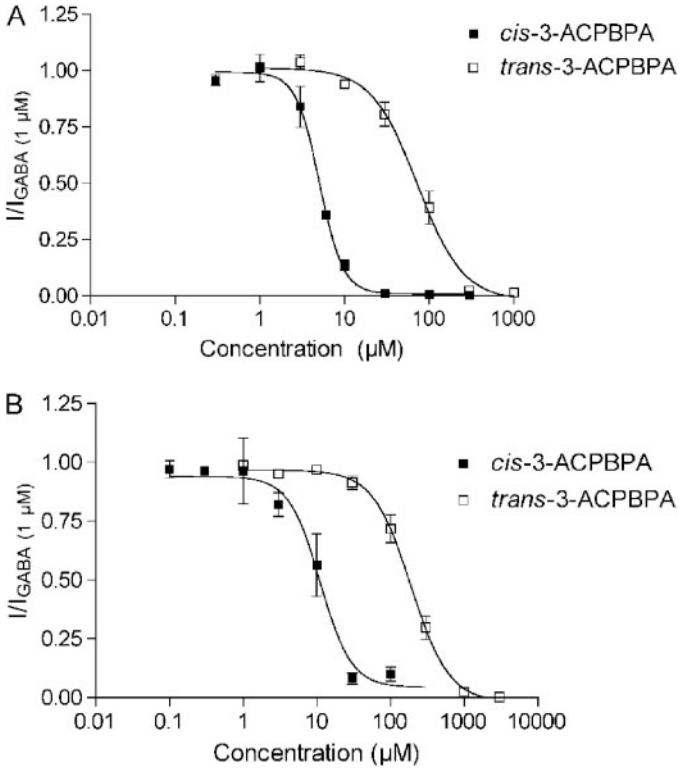

Fig. 3. Dose-response curves for *cis*- (□) and *trans*-3-ACPBPA (■) (A) in the presence of GABA (1 μM) at human ρ1 $GABA_C$ receptors expressed in *X. laevis* oocytes, clamped at −60 mV. Data are the mean ± S.E.M. Dose-response curves for *cis*- (□) and *trans*-3-ACPBPA (■) (B) in the presence of GABA (1 μM) at human ρ2 $GABA_C$ receptors expressed in *X. laevis* oocytes, clamped at −60 mV.

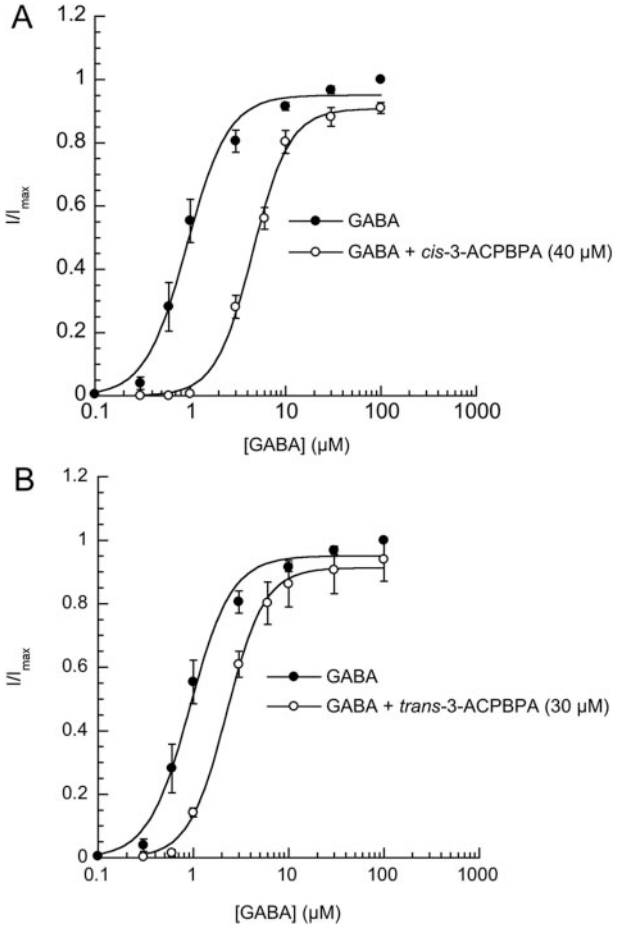

Fig. 4. Dose-response curves for GABA alone (●; $n = 6$) and GABA in the presence of 40 μM *cis*-3-ACPBPA (○; $n = 3$) (A) and GABA in the presence of 30 μM *trans*-3-ACPBPA (○; $n = 3$) (B) at human ρ1 $GABA_C$ receptors expressed in *X. laevis* oocytes and clamped at −60 mV. Data are the mean ± S.E.M. *cis*- and *trans*-3-ACPBPA seem competitive over the concentration tested.

cant decrease in the time taken to find the platform, and a statistically significant increase in the behavior score was observed with *trans*-3-ACPBPA (100 mg/kg) compared with vehicle control group (Table 4; Fig. 7; $p < 0.05$). A similar trend was observed with *trans*-3-ACPBPA (30 mg/kg), but this was not statistically significant ($p > 0.05$).

In contrast, on D1 scopolamine (1 mg/kg) decreased the number of rats that found the platform, induced a statistically significant increase in the time taken to find the platform, and there was a statistically significant decrease in the behavior score on trials 3 and 4, indicating impaired learning ability. On D2, scopolamine-treated rats showed no statistically significant changes on the number of rats, which found the platform, or the time taken to find the platform or on the behavior score by trials 2, 3, and 4. This indicates that by D2, scopolamine-treated rats quickly learned where the platform was located in the pool. This could be due to the pharmacokinetic properties of scopolamine, which has a half-life of 2.9 h (Benet et al., 1996).

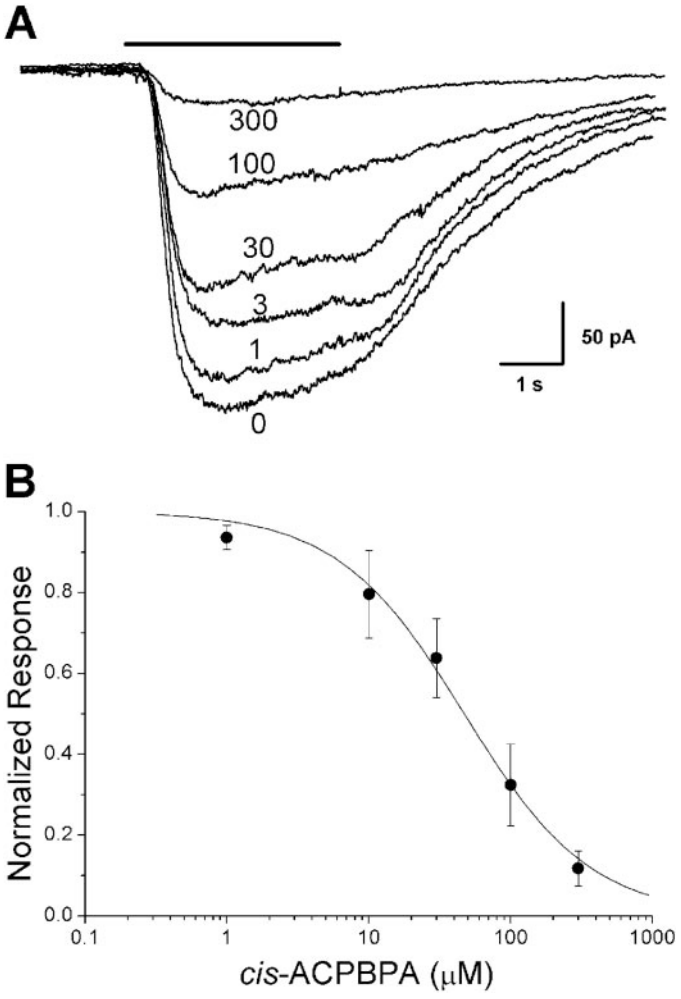

Fig. 5. A, examples of current responses from a rat retinal bipolar cell elicited by coapplication of GABA (10 μM) with various concentration of *cis*-3-ACPBPA (shown near each trace in micromolar). The responses were elicited in the presence of 100 μM bicuculline, which specifically inhibited the $GABA_A$ receptor activity on these cells. B, averaged dose-dependent inhibition of *cis*-3-ACPBPA on native $GABA_C$ receptor present on retinal bipolar cells. Data were normalized to response elicited by GABA (10 μM) alone and were fit with a Hill equation (continuous curve), with IC_{50} of 47 ± 4.5 μM and Hill coefficient of 0.97. Data show the means ± S.D. ($n = 6$).

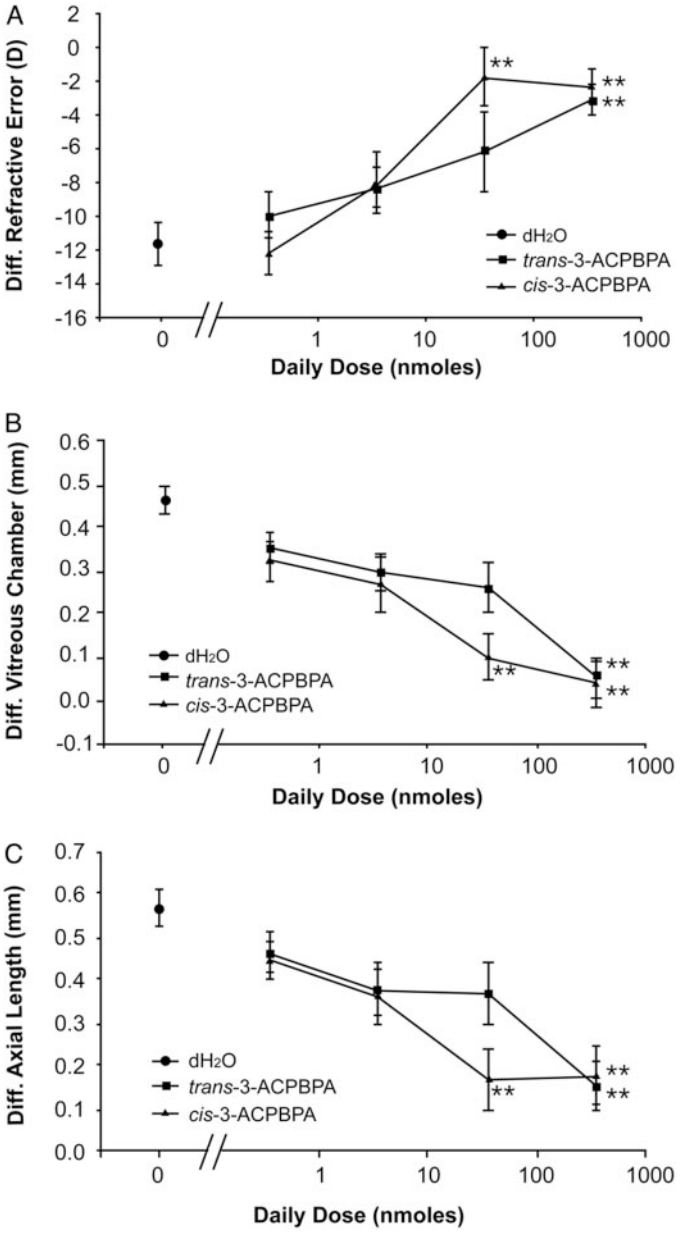

Fig. 6. Effect of *cis*-3-ACPBPA and *trans*-3-ACPBPA on the ocular effects of negative lens wear (−15 D). Interocular differences (mean ± S.E.) in refractive error (A), vitreous chamber (B), and axial length (C) are shown. Both drugs inhibited the ocular effects of negative lens wear in a dose-dependent manner. The two highest doses of *cis*-3-ACPBPA tested (35 and 350 nmol) significantly inhibited the myopia, vitreous chamber, expansion, and axial expansion compared with water-injected animals ($p < 0.005$ for all comparisons and both doses). For *trans*-ACPBPA, the effects of the −15 D lens were only significantly inhibited at the highest dose tested (350 nmol; $p < 0.005$ for all comparisons).

Discussion

cis- and *trans*-3-ACPBPA are restricted analogs of CGP36742 held between the C1 and C3 carbons (Fig. 1). This conformational restriction causes a dramatic change in pharmacology; although CGP36742 is almost equipotent at $GABA_B$ and $GABA_C$ receptors (Chebib et al., 1997), *cis*- and *trans*-3-ACPBPA are more potent and selective for the $GABA_C$ receptor. The cyclopentane moiety in *cis*- and *trans*-3-ACPBPA may reduce the available conformations that can

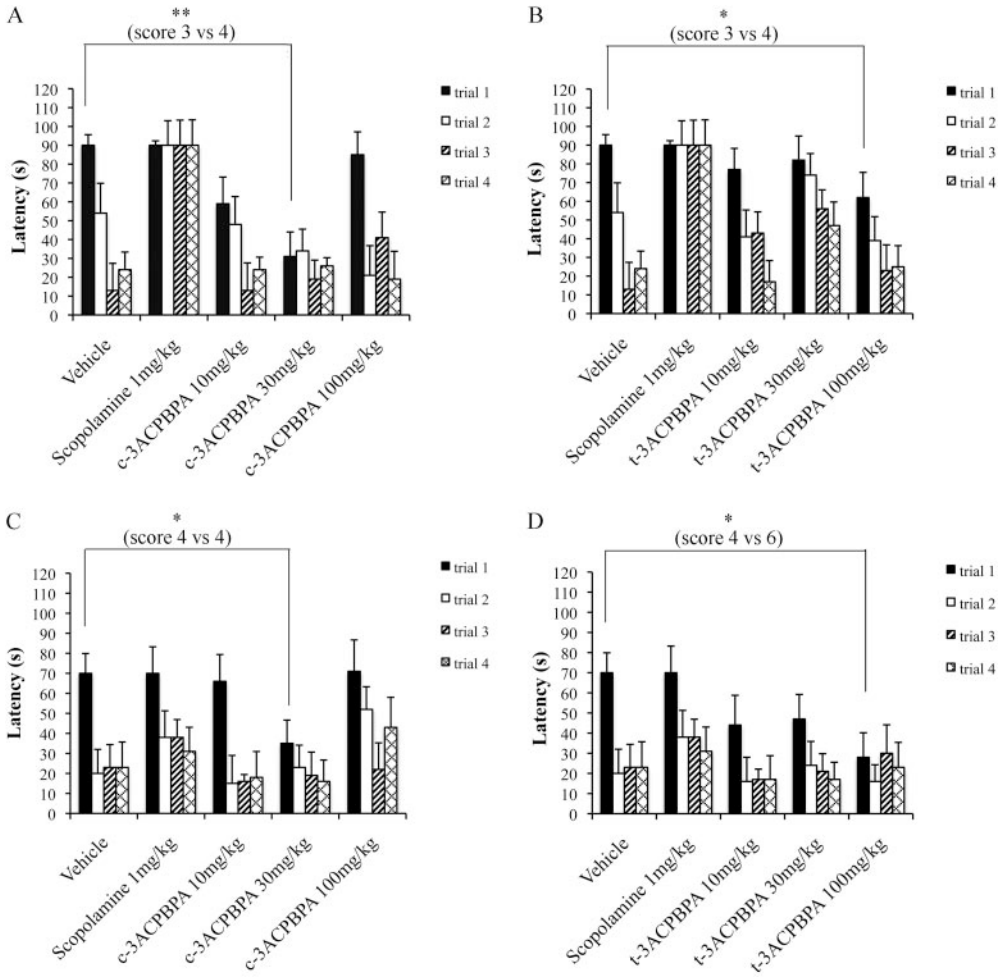

Fig. 7. Latency for rats to find the platform on D1 and D2 over four trials (median ± standard error of the median). Under the experimental conditions adopted (see *Materials and Methods*), scopolamine (1 mg/kg; A and B) reduced the number of rats that found the platform and showed a statistically significant increase in the time taken to find the platform and a statistically significant decrease in the behavior score on trials 3 and 4 of D1 (score of 3 for scopolamine versus 6 for vehicle). On D2, scopolamine (1 mg/kg; C and D) had no statistically significant effects on rat behavior. In contrast, "learning" was improved with *cis*-3-ACPBPA (30 mg/kg; $p < 0.01$, nonparametric Mann-Whitney U test) (A) and *trans*-3-ACPBPA (100 mg/kg; $p < 0.05$, nonparametric Mann-Whitney U test) (B) administered by the intraperitoneal route because these doses significantly decreased the time taken for the rat to locate the platform and produced a higher behavior score in the pool during trial 1 on D1 compared with vehicle control. On D2, rats dosed on D1 with *cis*-3-ACPBPA (30 mg/kg; $p < 0.05$, nonparametric Mann-Whitney U test) (C) and *trans*-3-ACPBPA (100 mg/kg; $p < 0.05$, nonparametric Mann-Whitney U test) (D) retained the ability to reach the platform significantly more quickly than vehicle and in the case of *trans*-3-ACPBPA (100 mg/kg), there was significant increase in the behavior score.

bind to either $GABA_A$ or $GABA_B$ receptors and favor those conformations binding to $GABA_C$ receptors.

Of the two new compounds studied, *cis*-3-ACPBPA was approximately 15 times more potent than the *trans*-analog at the ρ1 and ρ2 $GABA_C$ receptors, indicating that there are differences in how the compounds bind to the ligand-binding site. A recent model of the ligand-binding site of the human ρ1 $GABA_C$ receptor reported a likely molecular basis for agonist and antagonist actions (Abdel-Halim et al., 2008). Figure 8 uses this model to show *cis*- (Fig. 8A) and *trans*-3-ACPBPA (Fig. 8B) docked into the binding site: the *cis*-compound seems to have a stronger cation-π interaction compared with the *trans*-analog as the distance between the amine group of the ligand, and Tyr198, the amino acid involved in cation-π interactions (Lummis et al., 2005) is closer (distance, 3.1–4.4 Å for the *cis* versus 3.5–4.6 Å for the *trans*). This may provide a more favorable interaction at the ρ1 $GABA_C$ receptor compared with the *trans*-compound leading to improved affinity.

CGP36742 has been shown to improve cognitive performance in rodents and nonhuman primates in a wide range of learning and memory tasks via oral and intraperitoneal routes (Carletti et al., 1993; Mondadori et al., 1993) reaching phase II clinical trials for mild cognitive impairment and Alzheimer's disease (Bullock 2005). The mechanism by which SGS742 (CGP36742) improves memory is not completely known, and clinical studies attempt to link its effect to $GABA_B$ receptors despite its potency at $GABA_C$ receptors. In this study, we evaluated the effects of the conformationally restricted $GABA_C$-selective analogs of CGP36742, *cis*- and *trans*-3-ACPBPA, on learning and memory.

TABLE 4
Performance in the Morris water maze during trial 1 on days 1 and 2

Substance	Dose D1	Median Time to Reach Platform (D1, D2)	No. to Reach Platform	Behavior in Pool
	mg/kg	*S*		
Vehicle		90, 70	2, 7	3, 4
cis-3-ACPBPA	10	59, 71	5, 8	3, 4
	30	31**, 35*	7*, 9	4*, 4
	100	85, 71	5, 6	3, 4
trans-3-ACPBPA	10	77, 44	5, 8	3, 4
	30	82, 47	5, 8	3, 5
	100	61*, 28*	6, 9	4, 6*

** $p < 0.01$ and * $p < 0.05$ compared with vehicle, nonparametric Mann-Whitney U test, unilateral comparison.

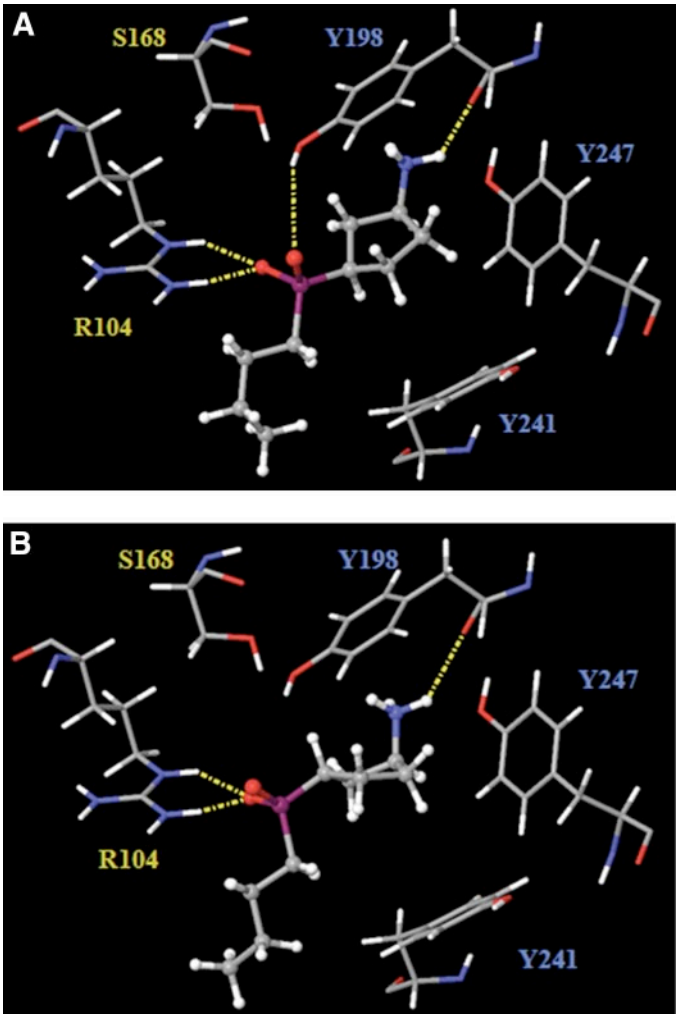

Fig. 8. *cis*-3-ACPBPA (ball and stick; A) and *trans*-3-ACPBPA (ball and stick; B) docked into the ρ1 $GABA_C$ receptor ligand-binding site. H-bonds between *cis*- and *trans*-3-ACPBPA and the binding site residues (stick) are highlighted (loop B, Tyr198; loop C, Tyr241 and Tyr247; loop D, Arg104; and loop E, Ser168). The distance between Tyr198 and the amine groups ranged from 3.1 to 4.4 Å and from 3.5 to 4.6 Å for the *cis*- and *trans*-compound, respectively.

On intraperitoneal administration, *cis*- (30 mg/kg) and *trans*-3-ACPBPA (100 mg/kg) treated rats tended to find the platform more quickly along with an increased number of rats that found the platform compared with control animals on days 1 and 2. Because the drug was given on day 1, it must be noted that an increase in activity of animals can also be related to a stimulant effect of the compounds and because of chance could lead to a decrease in the time the animals take to find the platform. However, the graduation of the behavior in the pool is important to differentiate between a stimulant effect on activity and an increase in learning capacity of the rat. Because there was a statistically significant increase in the behavior score on D1 for *cis*- and *trans*-3-ACPBPA, it indicates increased learning capacities.

On D2, rats treated with *cis*- (30 mg/kg) and *trans*-3-ACPBPA (100 mg/kg) tended to find the platform more quickly along with an increased number of rats, which found the platform compared with vehicle. However, in the case of *cis*-3-ACPBPA (30 mg/kg) the behavioral score was different to D1, indicating that memory may not be enhanced, whereas the behavioral score in the pool for *trans*-3-ACPBPA (100 mg/kg) was statistically significant and thus could facilitate learning and memory in adult rats.

Other $GABA_C$-selective antagonists, such as TPMPA, have been shown to enhance reinforced memory (Gibbs and Johnston, 2005) and alter sleep-waking behavior in rats (Arnaud et al., 2001). However, no systemically administered in vivo studies have been reported, indicating that TPMPA or related compounds (Murata et al., 1996; Krehan et al., 2003) do not cross the blood-brain barrier. Thus, we hypothesize that the $GABA_C$ receptor may play a role in learning and memory and that this receptor may, in part, be contributing to the learning and memory effects observed with CGP36742. However, it has been observed that on occasion, the $GABA_B$ and $GABA_C$ antagonist effects of CGP36742 neutralize each other as evidenced in sleep processes (Deschaux et al., 2006). Thus, to what extent $GABA_C$ receptors are involved in memory and learning processes of CGP36742 needs further investigation.

$GABA_C$ receptor mRNA and protein have been found in the rat hippocampus (Boue-Grabot et al., 1998; Alakuijala et al., 2006), an area of the brain associated with learning and memory. GABA-activated $GABA_C$ receptors in the CA1 area of the stratum pyramidale of adult rat hippocampus have been reported, following synaptic transmitter release after strong stimulation or under conditions of reduced GABA uptake, indicating the receptors are extrasynaptic being activated via GABA spillover (Alakuijala et al., 2006). Extrasynaptic receptors could influence the overall level of excitability of the postsynaptic neuron and function via tonic inhibition. As $GABA_C$ receptors have a high affinity for GABA (and therefore are activated by the low concentrations of the neurotransmitter that would be found outside the synapse) and a slow rate of desensitization, their physiology is consistent with such a role. Thus, selective $GABA_C$ antagonists may have a role in reducing tonic inhibition to possibly increase long-term potentiation.

$GABA_C$ receptors in the retina are clustered at distinct synapses to $GABA_A$ receptors (Euler and Wässle, 1998). These receptors prolong the duration of inhibition at mouse rod bipolar cell terminals both by direct activation and GABA spillover. The results suggest that inhibiting $GABA_C$ receptors increases the extent of neurotransmitter release from rod bipolar cells (Euler and Wässle, 1998).

Although the signal pathways in myopia formation have not been clearly delineated, there is convincing evidence for a pivotal role of the retina (Wallman, 1993). TPMPA has been shown to largely eliminate experimental myopia and reduce the associated axial elongation in both goggled (Stone et al., 2003) and negative lens wearing (Schmid et al., 2004) chick eyes. Like, TPMPA, *cis*- and *trans*-3-ACPBPA 1) reduced the refractive error, 2) inhibited the vitreous chamber elongation, and 3) reduced the axial length of the eye in a dose-dependent manner. Compared with TPMPA (Schmid et al., 2004), a lower dose of *cis*-3-ACPBPA (35 versus 70 nmol) and higher dose of *trans*-3-ACPBPA (350 versus 70 nmol) were required to inhibit a similar degree of lens-induced myopia (>80%). The differences in activity may reflect the ability of the compounds to diffuse within the vitreous to reach the retina or that the intraocular concentration of GABA is so high that it is displacing the antagonists from the receptor or that the subunits that make up the $GABA_C$ receptor in the retina is predominantly the combined ρ1 and ρ2 heteromeric receptor. Another explanation may

be due to the high soluble nature of the agents in aqueous solution and clearance of the compounds may be rapid reducing any potential accumulation in the eye. Good penetration of drugs through the vitreous requires the compounds to have both lipophilic and hydrophilic characteristics, which may be lacking in *cis*- and *trans*-3-ACPBPA. Whatever the reason for this discrepancy between the myopia data and the electrophysiological data, $GABA_C$ antagonists remain far more potent at inhibiting myopia progression than either $GABA_A$ or $GABA_B$ antagonists (Stone et al., 2003). Because CGP36742 has also been shown to inhibit lens-induced myopia to a similar degree and at a similar dose (280 nmol; Schmid et al., 2004) to *cis*- and *trans*-3-ACPBPA, it suggests that the effects of CGP36742 are most probably due to its $GABA_C$ rather than its $GABA_B$ receptor activity.

Song et al. (2005) proposed that the $GABA_C$ receptor behaves as a metabotropic receptor that links GABA signals and the retinoic acid pathway in the retina. Because retinoic acid has been implicated in controlling eye growth and myopia, the metabotropic action of the $GABA_C$ receptor could play a role in myopia formation. An alternative mechanism for the action of $GABA_C$ antagonists in myopia is the modification of ON/OFF retinal pathways (Schmid et al., 2005). Thus, $GABA_C$ antagonists may be useful in studying retinal mechanisms that modulate the growth of the eye and may provide leads to novel approaches to the inhibition of myopia. $GABA_C$ specific compounds with high potency and specificity are useful pharmacological tools to study mechanisms of myopia development.

In conclusion, this pharmacological evaluation of *cis*- and *trans*-3-ACPBPA at $GABA_A$, $GABA_B$, and $GABA_C$ receptors has contributed to the structure-activity relationship profiles for $GABA_C$ receptors. The discovery that *cis*- and *trans*-3-ACPBPA have weak effects at $GABA_A$ receptors, weak effects at $GABA_B$ receptors, but potent antagonist effects at $GABA_C$ receptors emphasizes some important pharmacological differences between these GABA receptor families. $GABA_C$ receptors may be of clinical and pharmacological interest as potential therapeutic targets for myopia and in enhancing cognition and managing memory-related disorders associated with schizophrenia and Alzheimer's disease (Johnston et al., 2003). Given the lower abundance, structural simplicity, and less widespread distribution of $GABA_C$ receptors in the central nervous system compared with $GABA_A$ receptors, $GABA_C$ receptors may be attractive central nervous system drug targets.

Acknowledgments

We are very grateful to the Department of Pharmacology, The University of Sydney, for managing and maintaining the *X. laevis* colony. H.-L.K. and H.A.-H. acknowledge the Faculty of Pharmacy Postgraduate Awards and the John Lamberton top-up scholarships, and R.J.K. acknowledges support from an Australian Postgraduate Award.

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